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SHORT COMMUNICATION

ITS2 rDNA VARIATION OF TWO BLACK WIDOW SPECIES, *LATRODECTUS MACTANS* AND *LATRODECTUS HESPERUS* (ARANEAE, THERIDIIDAE)

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ABSTRACT. The taxonomic status of two closely related species of *Latrodectus*, *L. mactans* and *L. hesperus*, has been debated for many years. Based on morphological characteristics and genitalia, some workers consider them as valid species and others as subspecies. This study was conducted to determine whether the internal transcribed spacers 2 (ITS2) of rDNA exhibit sequence differences between the two taxa that could delineate their taxonomic relationship. Individuals of *L. mactans* and *L. hesperus* from six populations were collected and identified based on morphological characteristics. The ITS2 rDNA of nine individuals was sequenced and analyzed. Results indicate that the minimal differences present in the ITS2 sequences are taxonomically insignificant.

Keywords: Theridiidae, *Latrodectus*, rDNA, internal transcribed spacer 2

The literature reveals conflicting information concerning the taxonomic status of two black widow species, *Latrodectus mactans* (Fabricius 1775) and *L. hesperus* Chamberlin & Ivie 1935 (Araneae, Theridiidae). Taxonomic work based on morphological characteristics has produced controversial conclusions. The two spider taxa were designated as subspecies by Levi (1959), and as separate species by Kaston (1970, 1978). For this study, the spiders were identified to species following the characteristics outlined by Kaston. Specimens of each species are on deposit in the Invertebrate Collection at Midwestern State University, Wichita Falls, Texas.

The rRNA genes have long been recognized as attractive markers for phylogenetic studies (Hillis & Dixon 1991). These genes are organized in clusters of repeated units, each of which consists of coding sequences, and several transcribed and non-transcribed spacer regions (NTS). The transcription units include the 18S, 5.8S, and 28S genes as well as external and internal transcribed spacers (ETS and ITS). Coding regions and spacers differ greatly in their rate of evolution, and hence the rDNA clusters have the potential to reveal phylogenetic relationships at many taxonomic levels. The level of divergence observed in the spacer regions is appropriate for detecting differences between specific individuals, which provides a potentially useful marker with which to study the relationships of populations and closely related species (Cerbah & Souza 1998; Hedin 1997; Vogler 1994). For example, Vogler (1994) separated tiger beetles (*Cicindela dorsalis*); Hedin (1997) separated cave spider populations and species (*Nesticus*); Harris & Crandall (2000) separated closely related species and populations of freshwater crayfishes (Decapoda, Cambaridae).

This preliminary study of ITS2 rDNA variation was conducted using individuals from three populations for each species. *Latrodectus mactans* individuals were collected in Texas from Wichita (34°00′N, 98°42′W), Brown (31°45′N, 99°00′W) and Kimble (30°29′N, 99°46′W) Counties. *Latrodectus hesperus* individuals were collected from Eddy County, New Mexico (32°52′N, 104°45′W), Brewster (29°33′N, 103°47′W) and Garza (33°10′N, 101°20′W) Counties of Texas. Genomic DNA was isolated from leg IV of each individual spider using QIAGEN DNAeasy Tissue Kit (Qiagen, Inc., Valencia, CA). The DNA concentration was measured using a UV spectrophotometer (Pharmacia, Ultraspec III).

The ITS2 region of rDNA was amplified using 5.8S primer (5′-GGGACGATGAAGAACGCAGC-

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Lat CAGCTGCGAGACTTGGTGTGAATTGCAGGACACATTGAGCACTGATTTTTCGAACGCGCA
Eno CAGCTGCGAGACT-GGTGTGAATTGCAGGACACATTGAGCACTGATTTTTCGAACGCGCA
    ************* ********************************************

Lat TTGCGCCCTCT-----TCCCCGGGGGCTCGCCTGTCTGAGGGTCGGATAAGACTTACCGA
Eno TTGCGGCCTCGGGTCCTGCCCGGGGCCTCGCCTGTCTGAGGGTCGGATAAGACTTGCAAA
    ***** ****       * ******* ***************************** *  *

Lat GGAGGGTTTCCCTCCCA-TTGGCTGCATCGGATGG---TGATCATCCGTCTGCCTAAGGT
Eno GGAAAGTTCGCTTTCCACTTGGCCGAACCGGATCGCTTTTGTGGTTCGCCGGCTTAAGGT
    ***  ***  * * *** ***** * * ***** *   *      * ** * ** ******

Lat CTTCGTCCGCGAATCCCCCTGGCCGAGAAGCGTCGCTCC---TCGAATAGGTCCAGCATA
Eno TTACG-----GATCCTCCCCGGCCGAGAAGCGTGGCTCCCACTTGACCAACGCTCGCGG-
     * **      **  * *** ************ *****   * **  *   *  **

Lat GAGGGAGAACGACTGA-TCGTCTCCCCGCCGACTGGAGCTGGAGCTGATGACGCAGAGAG
Eno GCTGGAGAA-GACTGAGTAGTTTCTCCGCTGAAGCGAGCGACGGGAGCGAGCGCAGAGAG
    *  ****** ****** * ** ** **** **   ****    *  *    *********

Lat TGTGCCGGGAAGAAGGGATGGTCGGATGGTCCGCTTGGGTGCTTTCGTGCTAATGCGTCC
Eno CTTGCCGAGGAGA-----------GATCCTCCAC--------------------GCGTGC
      ***** * ***           ***  *** *                    **** *

Lat CTCGTTCGTATAACGGCGTTGAACTACGTCCTGAATTGAGGGTTCGCAGCGAAAGGTCAC
Eno C-----------AC-----------------------------TGGCACGGTAAACTCAA
    *           **                             * ***  * **  ***

Lat GACTGATATTCATATCTGTCGACCTCAGATCAGACGAGATGACCCGCTGAATTTAA
Eno AA---------ATAT-TGTCGACCTCAGATCAGACGAGATGACCCGCTGAATTTAA
     *         **** ****************************************
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Figure 1.—Alignment of ITS2 regions from *Latrodectus* spp. and *Enoplognatha ovata*. Lat. = consensus sequence of eight *Latrodectus mactans* and *Latrodectus hesperus* individuals. Eno. = sequence of *E. ovata* (Araneae, Theridiidae). Underlined ITS2 sequences are flanked by 5.8S and 28S sequences. Asterisks indicate identical nucleotide positions. Alignment generated by CLUSTALW 1.4.

3′) and 28S primer (5′-TCCTCCGCTTATTGATATGC-3′) (Hillis et al. 1996). Two nanograms of spider genomic DNA were used for each reaction and PCR amplification was performed in 100 μL, using the following profile: 5 min at 94 °C, 40 cycles (1 min at 94 °C, 2 min at 45 °C, 1.5 min at 72 °C), and 7 min at 72 °C. The products were assessed by mini-gel electrophoresis using 5 μL aliquots. Successful amplifications generated a single 0.5 kb product. After purification, the PCR products were digested with *Eco*R I and *Hin*d III and ligated into pUC19 vector. The recombinant plasmids were transformed into *E. coli* DH5α cells. Sequences of both strands were determined by Northwoods DNA, Inc. (Center for Research and Innovation, 44526 CNTY Rd 3, Becida, MN 56678). Results were received as unprocessed nucleotide sequences and analyzed manually. Some visual adjustments were made. The individual sequences were aligned using CLUSTAL W 1.4 (Higgins & Sharp 1988) and consensus sequences were obtained.

First, the extent of the intergenic region between the 5.8S and 28S coding regions referred to as ITS2 was determined by similarity with the published sequences of *Enoplognatha ovata* (Araneae; Theridiidae) (Fig. 1) (Tan & Gillespie 1999). The length of the ITS2 region in different clones of *L. mactans* and *L. hesperus* is ~360 bp. Three individuals (LMW1, LMW2, LMW3) of the LMW population (*L. mactans* from Wichita County, Texas) were selected to analyze the variation in one population. For individual LMW3, four separate clones were sequenced to test variation within one individual. For the other populations, one individual from each was randomly selected for PCR amplification and one or more independent clones covering the ITS2 were sequenced from each of these individuals.

Five variable nucleotide positions were found in

	1	2	3	4
LMW1	C	T	T	T
LMW2	C	T	T	C
LMJ	T	T	A	T
LMB	T	A	T	T
LHM	T	A	T	T
LMW3	T	T	A	T
LHD	T	T	T	T
LHX	T	T	A	T

Figure 2.—Alignment of Variable ITS2 sites from eight *Latrodectus* individuals. Variable nucleotides occur at four positions within the *Latrodectus* consensus sequences in Figure 1: 1 = 124; 2 = 153; 3 = 227; 4 = 422. LMW = *L. mactans*, Wichita County, Texas; LMJ = *L. mactans*, Llano County, Texas; LMB = *L. mactans*, Brown County, Texas; LHM = *L. hesperus*, Eddy County, New Mexico; LHD = *L. hesperus*, Brewster County, Texas; LHX = *L. hesperus*, Garza County, Texas.

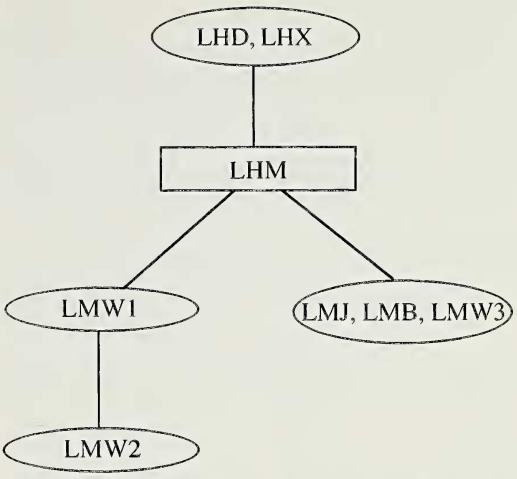

Figure 3.—Phylogenetic network of five haplotypes from ITS2 sequences of eight *Latrodectus* individuals. Open shapes, representing the haplotypes, indicate the included *Latrodectus* individuals. Analysis was performed by TCS (Clements et al. 2001).

the alignment of sequences from individual LMW3. Three variable nucleotide positions were found in the alignment of three individuals from Wichita County population of *L. mactans*. Three variable nucleotide positions were found in the alignment of three *L. mactans* populations. Only one variable nucleotide position was found among three populations of *L. hesperus*. The consensus sequences of eight individuals from six populations varied at four nucleotide positions (Fig. 2).

The phylogenetic analysis of the aligned sequences was performed as described by Templeton et al. (1992) using the TCS software package (Clement et al. 2000). ITS2 sequences from eight *Latrodectus* individuals collapsed into five haplotypes (Fig. 3). The three LMW individuals represented three different haplotypes on two different branches from a *L. hesperus* node. LMW3 and two *L. mactans* individuals from outside Wichita County represented a single haplotype. *Latrodectus hesperus* sequences collapsed into two haplotypes, one of which supported the two *L. mactans* branches.

Even though all the clones, individuals, populations and two taxa showed some level of sequence variation in the ITS2 region of rDNA, the separation between species was not well supported on these grounds. For *L. mactans*, variation within an individual is 1.4%, variation among individuals and that among populations are each 0.83%. The *L. hesperus* populations exhibit only 0.27% variation. The variation between two taxa is 0.83% and does not distinguish these two species. The same result was found in the ITS2 research of mosquitoes. Wesson et al. (1992) reported 0.46% variation within 10 clones of ITS2 from a single mosquito, *Aedes simpsoni*, while intraspecific variation in *A. aegypti* was only 1.17%. The differentiation even within a single individual may be caused by the existence of polymorphisms among repeat units of rDNA.

In a phylogenetic analysis of ITS2 sequence variations, *L. mactans* individuals from Wichita County represented as many haplotypes as the other five individuals, which included representatives of both *L. hesperus* and *L. mactans*. Furthermore, the three LMW haplotypes represented two distinct branches from LHM. Assuming that the Wichita County *L. mactans* individuals reflect a typical level of regional variation, ITS2 sequences do not offer a reliable means of distinguishing between *L. mactans* and *L. hesperus* populations.

Only those characters that are diagnostic for all individuals of an entire population can be used in the phylogenetic reconstruction of populations (Vogler 1994). Further exploration of the molecular taxonomy of these taxa will require additional data, including sequence comparisons of mtDNA, ITS1 DNA, plus other nuclear genes.

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